

REMARKS

This is responsive to the Office Action of 13 November 2003.

Amendments to the Claims

Without prejudice to the Applicant's ability to file continuing applications, applicants have cancelled all claims except for claims 9 and 10, which are directed to the compounds PAM-120 and PBM-100 respectively.

35 USC § 112 Rejection

The Examiner has rejected claims 7, 8, 14, 16, and 33-68 under 35 USC § 112, first paragraph. Applicants have previously cancelled claims 7 and 8. Applicants have cancelled claims 1 to 8 and 11 to 68 and submits that the Examiner's objection is now rendered moot.

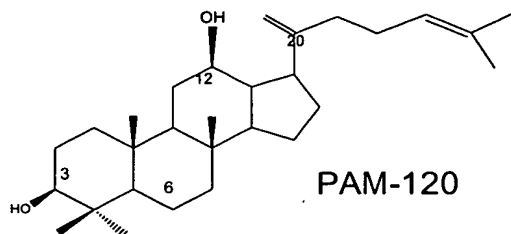
35 USC § 102(b) Rejection

The Examiner has rejected claims 1 and 2 as being anticipated by Park et al. Claims 1 and 2 are not directed to a compound disclosed in Park et al. Applicants have cancelled claims 1 to 8 and 11 to 68 and submits that the Examiner's objection is now rendered moot.

35 USC § 103(a) Rejection

The Examiner has rejected claims 1-3, 9, 14, and 16 under 35 USC § 103(a) as being unpatentable over Park et al., Yun et al., and Sun Wong Kwon et al. Applicants have cancelled claims 1 to 8 and 11 to 68. Therefore, the only claim which remains pending with respect to this rejection is claim 9. The Examiner has not rejected claim 10 under 35 USC § 103(a).

As discussed above, claim 9 is directed to the compound PAM-120 having the formula:



In light of the following, applicants submit that claim 9 is not obvious over Park et al., Yun et al., and Sun Wong Kwon et al.

Neither Sung Wong Kwon et al. nor Yun et al. disclose compounds which do not contain at least one sugar moiety. By contrast, the compounds of the present invention do not contain any sugar moieties. While the Examiner has argued that one skilled in the art would have been motivated to cleave the sugars from the disclosed compounds to obtain applicants' compound, as stated below, the combined disclosures of the cited art not only fail to motivate a person skilled in the art to do this but actually teach away from this. In addition, while Park et al. does disclose a compound (Qppd) which does not contain sugar moieties, the combined disclosures of the art do not provide any motivation to modify this compound to arrive at applicants' claimed compound as data present in the art itself (see Park et al.) show the unpredictability of such compounds. All of this is described in more detail below.

a) Sung Wong Kwon et al.

The Examiner has cited the article "Liquid chromatographic determination of less polar ginsenosides in processed ginseng" by Sung Won Kwon et al., Journal of Chromatography A, 921 (2001) 335-339 ("Kwon et al."). At page 6 of the Office Action, under paragraph 1, "Determining the scope and contents of the prior art", the Examiner contends that Kwon et al. "teaches the ginsenoside Rg3 inhibition on multi drug resistance, see the entire document especially page 336 where anticancer activity of the ginsenosides are disclosed."

With due respect, applicants submit that Kwon et al. does not teach the inhibition of multidrug resistance by Rg3. Page 336 of Kwon et al. does not make any reference to the inhibition of multidrug resistance by Rg3. Page 336 only refers to ginsenoside structures.

On page 335 of Kwon et al., in the Introduction, it is disclosed that "Ginsenoside Rg3 showed strong *vasorelaxation activity and anti-platelet aggregation activity*." These activities are not in any way related to multidrug resistance inhibition activities. Moreover, Kwon et al. does not disclose any experimental data or present any examples which demonstrate that ginsenoside compounds have anticancer activity. Accordingly, Kwon et al. does not provide any guidance to a person skilled in the art which would lead that person to the compound claimed in claim 9.

Furthermore, on page 6 of the Office Action, under paragraph 2 "Ascertaining the differences between the prior art and the claims at issue", the Examiner asserts that the compound claimed (i.e. PAM-120 in claim 9) differs from the references only by claiming a different position of double bond in the side chain at 17-position. With respect, applicants submit that PAM-120 as claimed in claim 9 differs from the structures in Kwon et al. by more than the position of the double bonds in the side chain. None of the structures disclosed on page 336 of Kwon et al. are positional isomers of PAM-120. All of the compounds disclosed in Kwon et al. have at least one sugar moiety. PAM-120 does not contain any sugar moieties.

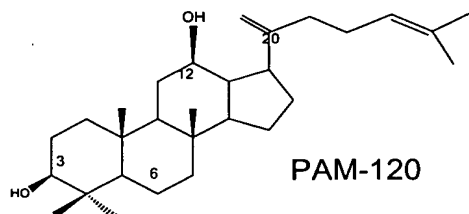
On page 7 of the Office Action, the Examiner contends that "Cleavage of sugar from saponins to get sapogenins is conventional and is known to one skilled in the art." However, applicants submit that it is not in fact conventional and known in the art to cleave sugars from saponins. Arguably, Kwon et al. teaches away from the claimed compounds because all of the compounds in Kwon et al. contain sugars, and Kwon et al. does not disclose how a person skilled in the art would cleave sugars from saponins. There is no motivation in Kwon et al. that would lead a person skilled in the art to cleave the sugars from saponins to obtain the claimed compounds. Moreover, there is no teaching in Kwon et al. that would lead a person skilled in the art to think that a sapogenin compound without sugar moieties would necessarily have any activity compared to a saponin compound with sugar moieties. Conversely, applicants submit that it is known in the art that the activity of sapogenins, saponins, and ginsenosides cannot be predicted from one compound to another. Accordingly, applicants submit that Kwon et al. does not teach the claimed compound PAM-120, and claim 9 is not obvious in light of Kwon et al. Applicants request respectfully the withdrawal of the citation of Kwon et al. against claim 9.

b) Yun et al.

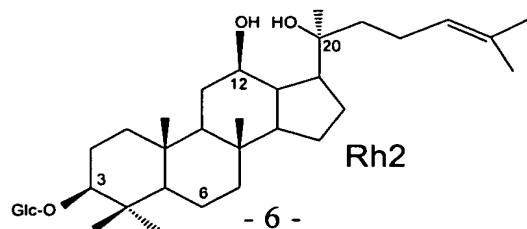
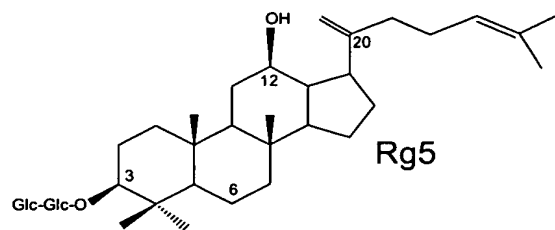
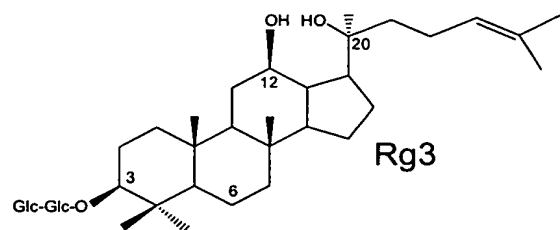
The Examiner has cited the article "Anticarcinogenic Effect of Panax Ginseng C.A Meyer and Identification of Active Compounds" by Yun et al., J. Korean Med. Sci, 2001:16(Suppl):S6-18 ("Yun et al."). The Examiner contends that Yun et al. teaches that the ginsenosides Rg3, Rg5, and Rh2 are active anticarcinogenic compounds and that "they are said to prevent cancer either singularly or synergistically, see the abstract, Tables 1-8, Fig. 1-3 and compounds on page S 13." Furthermore, the Examiner contends that the claimed compound (namely PAM-120) differs from the references by claiming a different position of double bond in the side chain at 17-position.

Again, applicants submit respectfully that PAM-120 is not a positional isomer of the compounds Rg3, Rg5, and Rh2. In particular, PAM-120 differs from these compounds because PAM-120 does not contain sugar moieties.

PAM-120 has the following structure:



Rg3, Rg5, and Rh2 have the following structures:



PAM-120 is clearly different from Rg3, Rg5, and Rh2 and cannot be a positional isomer of Rg3, Rg5, and Rh2 due to the presence of the sugar moieties at the 3-position on Rg3, Rg5, and Rh2.

Furthermore, as discussed above, the Examiner contends that "Cleavage of sugar from saponins to get sapogenins is conventional and is known to one skilled in the art." Again, applicants submit that Yun et al. does not teach the cleavage of sugars from saponins. In fact, Yun et al. arguably teaches away from the claimed compounds as the compounds in Yun et al. all have sugar moieties. There is no motivation in Yun et al. to cleave sugars from saponins to obtain the claimed compounds. Moreover, there is no teaching in Yun et al. that would lead a person skilled in the art to think that a sapogenin compound without sugar moieties would necessarily have any activity compared to a saponin compound with sugar moieties. Conversely, applicants submit that it is known in the art that the activity of sapogenins, saponins, and ginsenosides cannot be predicted from one compound to another. Accordingly, applicants submit that Yun et al. does not teach the claimed compound PAM-120, and claim 9 is not obvious in light of Yun et al. Applicants request respectfully the withdrawal of the citation of Yun et al. against claim 9.

c) Park et al.

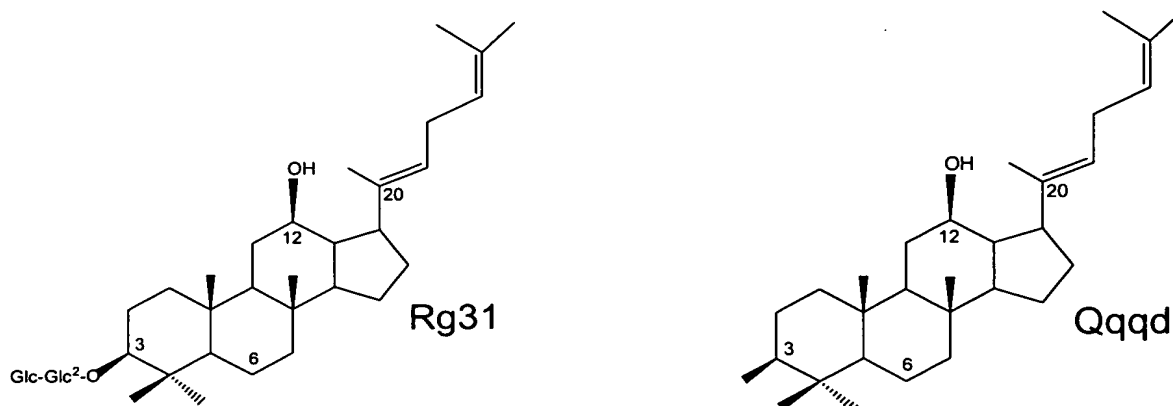
The Examiner has cited the article "Effects of Ginseng Saponin on Modulation of Multidrug Resistance" by Park et al., Arch. Pharm. Res. Vol. 19, No. 3, pp. 213-218, 1996 ("Park et al.") against claim 9. At page 6 of the Office Action, the Examiner asserts that Park et al. "teach ginseng saponins for multidrug resistance" and the use of saponins as antitumor agents. Furthermore, the Examiner contends that the claimed compound (namely PAM-120) differs from the references by claiming a different position of double bond in the side chain at 17-position. The Examiner also contends that "Cleavage of sugar from saponins to get sapogenins is conventional and is known to one skilled in the art."

With respect, applicants submit that Park et al. does not generally teach that saponins are useful as antitumor agents and for reversing multidrug resistance in cancer cells. On the contrary, Park et al. demonstrates that different saponins, sapogenins, and ginsenosides have divergent anticancer activities, even if the saponins, sapogenins, and ginsenosides only differ by small structural differences. Park et al. also demonstrates that anticancer

activity in one compound cannot be predicted by comparing the compound to structurally similar compounds.

Tables II and III on page 216 of Park et al. demonstrates that different ginsenoside compounds have very different and unpredictable anticancer activities. In Table II, the data indicates that compounds without sugar moieties, such as protopanaxadiol and protopanaxatriol, are either very weak in cytotoxicity and reversal of multidrug resistance, or have no effects at all (for example, see activity of compounds Qppd and Qppt in Table II). Some compounds with one sugar moiety do not exhibit any cytotoxicity (for example 20(R)-Rh2 and Rh3), but others do (for example 20(R)-Rh1, 20(S)-Rh1, 20(S)-Rh2, and Rh4). Compounds with two or more sugar moieties also demonstrate a lack of predictability in anticancer activity. For example, 20(R)-Rg3 appears to have no cytotoxicity, while 20(S)-Rg3 seems to have some cytotoxic and multidrug resistance reversal properties. 20(S)-Rg2 and 20(R)-Rg2 do not demonstrate any cytotoxic or multidrug resistance inhibition properties, but Rg31 appears to have some cytotoxic and multidrug resistance reversal properties. This reinforces the applicants' submission that cytotoxic properties cannot be readily predicted from one saponin, sapogenin, or ginsenoside to another.

Applicants draw the Examiner's attention to the activities of the compounds Rg31 and Qppd listed in Tables II and III of Park et al. Rg31 and Qppd have the following structures:



Rg31 and Qppd are similar in structure but for the presence of the glycon moieties in Rg31. Table III illustrates that Rg31 has some cytotoxic and multidrug resistance reversal activity, while Qppd has none (See Table II). In this example, it appears that cytotoxic and multidrug resistance reversal activity is dependent on the presence of sugar moieties. Applying this reasoning, it would appear that the claimed compound, PAM-120, which does not have sugar moieties, would not have anticancer or multidrug resistance reversal activity. On the contrary, applicants have discovered that PAM-120 surprisingly has very strong anticancer activities. This result would not have been predicted by Park et al. In fact, Park et al. arguably teaches away from the claimed compound.

Therefore, although the Examiner contends that "Cleavage of sugar from saponins to get sapogenins is conventional and is known to one skilled in the art," applicants submit that there is no teaching or motivation in the cited art that would lead a person skilled in the art to the claimed compound PAM-120. The cited art *teaches away* from the invention. In fact, at page 216 of Park et al., in the last sentence of the first paragraph on the page, it is disclosed that "*removal of a glucoside portion of the ginsenosides Rh3 (9) and Rh4(11) failed to inhibit Pgp-MDR.*" Accordingly, applicants submit that claim 9 is not obvious in light of Park et al. and respectfully requests withdrawal of the citation of Park et al. against claim 9.

d) Side-by-Side Experiments to Compare the Activity of PAM-120 to the Activity of Prior Art Compounds

Applicants have also conducted side-by-side experiments to compare the activity of the claimed compound PAM-120 (and PBM-100) to prior art compounds Rg3 and Rh2. The results of the experiments are submitted pursuant to 37 C.F.R. 1.132 in the form of the Affidavit of Mr. Dong Huang, one of the inventors of the claimed invention. Mr. Huang's Affidavit is attached hereto as Appendix "A".

Applicants do not admit that the compounds Rg3 and Rh2 are similar to PAM-120 (or PBM-100). On the contrary, applicants submit that PAM-120 (and PBM-100) are completely different compounds from Rg3 and Rh2. No comparison can be drawn between the compounds, and no activity can be predicted by comparing the structures of the compounds. However, for completeness, applicants submit the results of the side-by-side

experiments in Mr. Huang's Affidavit to demonstrate that PAM-120 (and PBM-100) are significantly more effective than the compounds Rg3 and Rh2.

Table 1 from Mr. Huang's Affidavit (which is reproduced below) illustrates that PAM-120 (and PBM-100) are more significantly more effective at reducing cancer cell viability than Rg3 and Rh2.

Compound (25 uM)	Absorbency of stained cells (M +/-SD)	Viability (%)
Blank control	0.368 +/- 0.069	100.00
Rg3	0.298 +/- 0.071	80.98
Rh2	0.278 +/- 0.030	78.49
PAM-120	0.220 +/- 0.051	62.08
PBM-100	0.223 +/- 0.040	62.72

Table 1: Viability of H460 Lung Cancer Cells in the Presence of 25uM Rg3, Rh2, PAM-120 and PBM-100.

The results in Table 1 illustrate that H460 lung cancer cells are significantly less viable in the presence of PAM-120 and PBM-100 than either Rg3 or Rg2. Cells treated with PAM-120 and PBM-100 were nearly 25% less viable than cells treated with Rg3, and nearly 20% less viable than cells treated with Rh2. Therefore, PAM-120 and PBM-100 have much greater cytotoxic effects on lung cancer cells than either Rg3 or Rh2.

Table 2 from Mr. Huang's Affidavit (which is reproduced below) illustrates that PAM-120 (and PBM-100) are far more effective at inhibiting breast cancer cells at significantly lower concentrations than Rg3 and Rh2.

Compound	IC ₅₀ (ug/mL)
Rg3	67.5 +/- 9.0
Rh2	35.2 +/- 4.3
PAM-120	<10
PBM-100	15.3 +/- 2.3

Table 2: IC50 Values of Compounds PAM-120, PBM-100, Rg3, and Rh2 Against MCF7r Breast Cancer Cells.

PAM-120 has an IC50 nearly 7 times less than the IC50 of Rg3 and nearly 4 times less than the IC50 of Rh2. PBM-100 has an IC50 nearly 4.5 times less than the IC50 of Rg3 and nearly 2.5 times less than the IC50 of Rh2. Therefore, PAM-120 (and PBM-100) are significantly more effective against breast cancer cells at a much lower concentration than either Rg3 or Rh2.

Table 3 from Mr. Huang's Affidavit (which is reproduced below) illustrates that PAM-120 (and PBM-100) are far more effective at inhibiting melanoma cells at significantly lower concentrations than Rg3 and Rh2.

Compound	IC ₅₀ (ug/mL)
Rg3	30.2 +/- 4.6
Rh2	28.1 +/- 4.9
PAM-120	<10
PBM-100	<10

Table 3: IC50 Values of Compounds PAM-120, PBM-100, Rg3, and Rh2 Against B16 Melanoma Cells.

PAM-120 and PBM-100 have IC50 values nearly 3 times lower than the IC50 values of Rg3 and Rh2. Therefore, PAM-120 (and PBM-100) are significantly more effective against melanoma cells at a much lower concentration than either Rg3 or Rh2.

Applicants submit that these results demonstrate that PAM-120 (and PBM-100) are consistently and significantly more effective than Rg3 and Rh2 at inhibiting lung, breast, and melanoma cancer cells. Moreover, Tables II and III in Park et al. indicate that of the compounds tested by Park et al., Rg3 and Rg31 demonstrate higher levels of cytotoxic and multidrug resistance inhibition activity than the other compounds, and Rg3 and Rg31 have similar activities. However, PAM-120 (and PBM-100), as discussed above, have far

superior cancer cell inhibition properties over Rg3. Therefore, PAM-120 (and PBM-100) logically also have superior cancer cell inhibition properties over Rg31. PAM-120 (and PBM-100) are surprisingly more efficient compounds whose activities could not have been predicted from the cited prior art.

In addition, applicants submit that Table 8 on page S14 of Yun et al. compares the activity of Rg3 and Rg5. According to Table 8 of Yun et al., Rg3 and Rg5 appear to have similar anticarcinogenic activity. On page S13 of Yun et al., second to last paragraph it is disclosed that "Rg3 showed statistically significant decrease (22.2%) in lung tumor incident (46.7%; $p < 0.05$), whereas Rg5 and BP had biologically significant incidence (45.0% and 25.0% decrease)($p < 0.05$)(Table 8)." Since Rg3 and Rg5 have similar anticancer activities, and PAM-120 (and PBM-100) have superior cancer inhibition properties over Rg3, applicants submit that PAM-120 (and PBM-100) logically also have superior cancer inhibition properties over Rg5. PAM-120 (and PBM-100) are significantly superior compounds whose activities are not predictable based upon the prior art compounds.

In light of the foregoing, applicants submit that claim 9, which is directed to the compound PAM-120, is not obvious in light of the prior art of Kwon et al., Yun et al., and Park et al. Applicants request respectfully withdrawal of the citation of these references from this application.

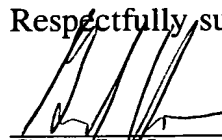
35 USC § 101 Rejection - Provisional Double Patenting Rejection

The Examiner has provisionally rejected claims 1-3, 9, 10, 14, and 16 for claiming the same invention as that of claims 1-3, 5, 7-10, 16-23, and 27-34 of copending Application No. 09/982,018. Applicants have cancelled claims 1 to 8 and 11 to 68. Accordingly, the rejection of claims 9 and 10 remain outstanding.

Applicants submit that claims 1-3, 5, 7-10, and 16-23 have been cancelled in copending Application No. 09/982,018. Claims 27-34 in the copending application are directed to **methods of making sapogenin compounds**, **not** to the sapogenin compounds themselves. Therefore, copending Application No. 09/982,018 does **not** contain claims directed to the same subject matter as claims 9 and 10. Accordingly, applicants submit that claims 9 and 10 are in condition for allowance.

In light of the foregoing, applicants submit that claims 9 and 10 are in condition for allowance, which is respectfully requested.

Respectfully submitted,



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Attorney for Applicants

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GJY

APPENDIX "A"

The Affidavit of Mr. Dong Huang, pursuant to 37 C.F.R. 1.132 is attached hereto.



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Paper No.: _____

IN THE UNITED STATES PATENT & TRADEMARK OFFICE

Inventor(s): HUANG, Dong; QI, Dong Feng
Title: NOVEL AGLYCON DAMMARANE SAPOGENINS, THEIR USE AS
ANTI-CANCER AGENTS, AND A PROCESS FOR PRODUCING SAME
Serial No.: 09/910887
Filed: 24 July 2001
Examiner: Qazi, Sabiha Naim Art Unit: 1616
Date: 10 May 2004

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Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

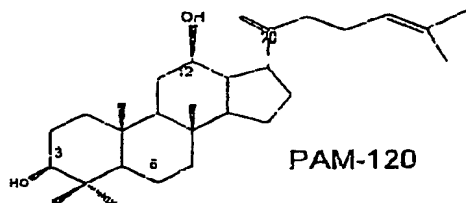
Dear Sir:

AFFIDAVIT UNDER RULE 1.132

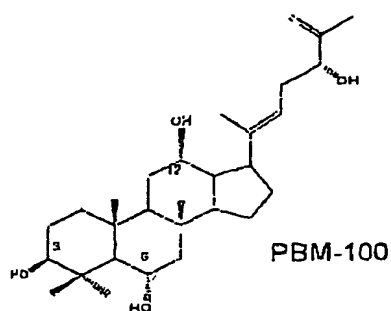
I, Dong Huang, of 16788 102 Avenue, Surrey, British Columbia, Canada, V4N 4X2,
MAKE OATH AND SAY AS FOLLOWS:

1. I have personal knowledge of the matters sworn to herein, except where the matters are stated to be based on information and belief, in which case I believe them to be true.
2. I am a co-inventor of the invention described and claimed in US Patent Application Serial No. 09/910887.
3. I hold a Bachelor of Science degree from the University of Beijing in China.
4. I have over 20 years of experience in the fields of botany chemistry research and ginsenoside drug development.
5. I have conducted side-by-side experiments to compare the efficacy of the compounds PAM-120, PBM-100, Rg3, and Rh2.

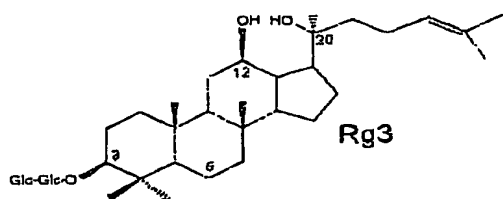
6. PAM-120 has the following formula:



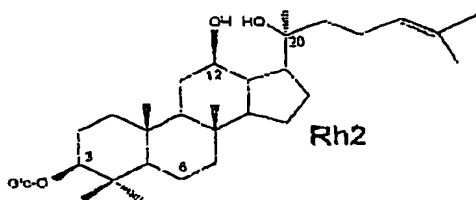
PBM-100 has the following formula:



Rg3 has the following formula:



Rh2 has the following formula:



7. I conducted experiments to compare the efficacy of PAM-120, PBM-100, Rg3, and Rh2 against lung cancer cells in the following manner. Compounds PAM-120, PBM-100, Rg3, and Rh2 were obtained from Pegasus Pharmaceuticals Group Inc. Human non-small-cell H460 lung cancer cells were seeded at 3×10^4 cells/well in a 96-well plate in DMEM medium supplemented with 10% fetal bovine serum and incubated at 37°C with 5% CO₂. The cells were treated with isolated ginsenoside Rg3, Rh2, PAM-120, and PBM-100 at a fixed dose of 25 uM. The cytotoxic effects of the compounds on the lung cancer cells were measured by determining the viability of the cells. Cell viability was measured using the MTT (3-(4,5-dimethylthiazol-2-yl) -2,5-diphenyltetrazolium bromide) assay method (Denizot and Kang, J. Immunol. Meth. 89:271-277 (1986); Carmichael et al., Cancer Res. 47:936-942 (1987)) 24 hours following treatment. Cell viability is measured by determining the absorbency of stained cells. Non-viable cells have lower absorbency compared to viable cells. Table I shows the viability of H460 lung cancer cells in the presence of the compounds Rg3, Rh2, PAM-120, and PBM-100 at 25 uM.

Compound (25 uM)	Absorbency of stained cells (MTT, SD)	Viability (%)
Blank control	0.368 +/- 0.069	100.00
Rg3	0.298 +/- 0.071	80.98
Rh2	0.278 +/- 0.030	78.49
PAM-120	0.220 +/- 0.051	62.08
PBM-100	0.223 +/- 0.040	62.72

Table 1: Viability of H460 Lung Cancer Cells in the Presence of 25uM Rg3, Rh2, PAM-120 and PBM-100.

8. The results in Table 1 illustrate that H460 lung cancer cells are significantly less viable in the presence of PAM-120 and PBM-100 than either Rg3 or Rh2. Therefore, PAM-120 and PBM-100 have greater cytotoxic effects than either Rg3 or Rh2.

9. I conducted experiments to compare the efficacy of PAM-120, PBM-100, Rg3, and Rh2 against breast cancer cells in the following manner. Compounds PAM-120, PBM-100, Rg3, and Rh2 were obtained from Pegasus Pharmaceuticals Group Inc. Human drug resistant MCF7r breast cancer cells were seeded at 3×10^4 cells/well in a 96-well plate in DMEM medium supplemented with 10% fetal bovine serum and incubated at 37°C with 5% CO_2 . The cells were treated with isolated ginsenoside Rg3, Rh2, PAM-120 and PBM-100 at various concentrations. The IC50s of the compounds Rg3, Rh2, PAM-120, and PBM-100 were determined using standard methods. IC50 is the concentration of a compound needed to reduce the growth of a population of cells by 50%. The IC50s of the compounds are shown in Table 2.

Compound	IC ₅₀ (µg/mL)
Rg3	67.5 +/- 9.0
Rh2	35.2 +/- 4.3
PAM-120	<10
PBM-100	15.3 +/- 2.3

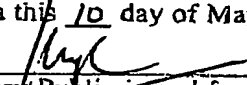
Table 2: IC50 Values of Compounds PAM-120, PBM-100, Rg3, and Rh2 Against MCF7r Breast Cancer Cells.

10. The results in Table 2 illustrate that PAM-120 and PBM-100 have significantly lower IC50 concentrations than Rg3 and Rh2. PAM-120 has an IC50 nearly 7 times less than the IC50 of Rg3 and nearly 4 times less than the IC50 of Rh2. PBM-100 has an IC50 nearly 4.5 times less than the IC50 of Rg3 and nearly 2.5 times less than the IC50 of Rh2. Therefore, PAM-120 and PBM-100 are effective at inhibiting MCF7r breast cancer cells at significantly lower concentrations than either Rg3 or Rh2.
11. I conducted experiments to compare the efficacy of PAM-120, PBM-100, Rg3, and Rh2 against melanoma cells in the following manner. Compounds PAM-120, PBM-100, Rg3, and Rh2 were obtained from Pegasus Pharmaceuticals Group Inc. Mouse B16 melanoma cells were seeded at 3×10^4 cells/well in a 96-well plate in DMEM medium supplemented with 10% fetal bovine serum and incubated at 37°C with 5% CO_2 . The cells were treated with isolated ginsenoside Rg3, Rh2, PAM-120 and PBM-100 at various concentrations. The IC50s of the compounds Rg3, Rh2, PAM-120, and PBM-100 were determined using standard methods. The IC50s of the compounds are shown in Table 3.

Compound	IC ₅₀ (µg/ml)
Rg3	30.2 +/- 4.6
Rh2	28.1 +/- 4.9
PAM-120	<10
PBM-100	<10

Table 3: IC₅₀ Values of Compounds PAM-120, PBM-100, Rg3, and Rh2 Against B16 Melanoma Cells.

12. The results in Table 3 illustrate that both PAM-120 and PBM-100 have IC₅₀ values nearly 3 times lower than the IC₅₀ values of Rg3 and Rh2. Therefore, PAM-120 and PBM-100 are effective at inhibiting melanoma cells at significantly lower concentrations than Rg3 and Rh2.

SWORN before me at the
city of SURREY, in the
Province of British Columbia,
Canada this 10 day of May, 2004

A Notary Public in and for the
Province of British Columbia,
Canada. My Commission is for life.


Dong Huang

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